IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT Application of
Berscheid, et. al.

Group Art Unit: 1621

Application Serial No. 08/860,007

Examiner: Shippen

Filed: August 4, 1997

For: BIOCIDAL ALCOHOLS, THEIR PRODUCTION AND THEIR USE

* * * * *

<u>RULE 132 DECLARATION</u>

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Ralf Berscheid declare and state as follows:

2. I am currently employed by Aesculap AG & Co. KG. My present position and title are Vice President Aesculap, Service Systems.

3. I have been continuously employed since 1989. During that time I have held the following positions:

<u>see attachment 2</u>.

4. I am the author or co-author of many articles related to the field of biocides. A list of these articles is shown in Attachment 1.

5. I have read and understood the present patent application. I have also read and understood the Office Actions and the cited prior art in the present patent application.

6. I performed or supervised the experiments in the paper entitled "Results of Screening Biocidal Alcohols" filed in the present application on July 17, 2000, copy attached herewith.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By

Date: 22.9.2003

Attachment 1

Publications:

F. Vögtle, R. Berscheid, W. Schnick, „Inclusion of Acetonitrile in a Macrobicyclic Host Molecule"; *J. Chem. Soc., Chem. Commun.* **1991**, 414-416.

R. Berscheid, M. Nieger, F. Vögtle, „Orientational Selectivity for the Inclusion of Acetonitrile in Tailor-made Macrobicyclic Host Molecules"; *J. Chem. Soc., Chem. Commun.* **1991**, 1364-1366.

R. Berscheid, F. Vögtle, „Concave Dyestuffs: A Triply Bridged Triphenylmethyl Dication"; *Chem. Ber.* **1992**, 58-62.

R. Berscheid, I. Lüer, Ch. Seel, F. Vögtle, „Molecules with Large Cavities – Selective Complexation of Inorganic and Organic Guests", in V. Balzani (Hrsg.): *Supramolecular Chemistry* (*NATO ASI Ser., Ser. C, Bd. 371*) **1992**, 71-86.

R. Berscheid, M. Nieger, F. Vögtle, „Mehrfach verbrückte Triphenylmethane"; *Chem. Ber.* **1992**, 1687-1695.

R. Berscheid, M. Nieger, F. Vögtle, „Konkave Farbstoffmoleküle vom Triphenylmethan-Typ"; *Chem. Ber.* **1992**, 2539-2552.

H. Eggensberger, R. Berscheid, „Antimikrobielle Wirkung S-substituierter Mercaptobenzthiazol-Derivate"; *Seife Öle Fette Wachse* **1993**, 581-584.

H. Eggensberger, R. Berscheid, „Herstellung und Verwendung von Derivaten des Thiosemicarbazids und Thiocarbazids als antimikrobielle Wirkstoffe"; *Seife Öle Fette Wachse* **1994**, 286-290.

R. Berscheid, H. Eggensperger, W. Beilfuß, S. Behrends, B. Puchstein, „Biozide Alkohole, ihre Herstellung und Verwendung"; *Off.leg. DE 4447361 A1*, **21.12.1994.**

Attachment 2

Employments:

01.02.1989 – 31.01.1991	"Fonds der Chemischen Industrie" Scholarship (German industrial scholarship), Bonn, Germany
01.02.1991 – 31.03.1992	Assistant lecturer within the study group of Prof. Dr. Fritz Vögtle, University of Bonn, Germany
01.07.1992 – 31.12.1992	Trainee, Schülke & Mayr GmbH, Norderstedt, Germany
01.01.1993 – 31.12.1994	Team Leader Product Development Antidermatica, Schülke & Mayr GmbH, Norderstedt, Germany
01.01.1995 – 31.12.1997	Associate, McKinsey & Company, Inc.
01.01.1998 – 31.12.1999	Engagement Manager, McKinsey & Company, Inc.
01.01.2000 until now	Vice President Aesculap Service Systems, Aesculap & Co. KG, Tuttlingen, Germany
01.07.2000 until now	CEO SteriLog GmbH, Tuttlingen, Germany

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT Application of
Berscheid, et. al.

Group Art Unit: 1621

Application Serial No. 08/860,007

Examiner: Shippen

Filed: August 4, 1997

For: BIOCIDAL ALCOHOLS, THEIR PRODUCTION AND THEIR USE

* * * * *

July 17, 2000

Results of Screening Biocidal Alcohols

0. Test method

To obtain comparable data the alcohols were measured within a **standard formulation** of

Rewopal MPG 40		25.0 g
aromatic alcohol		10 mmol
Water (demin.)	ad	100.0 g

Lactic acid for adjusting the pH value to 7.0 (which is important for the dilution process for the antimicrobicidal tests)

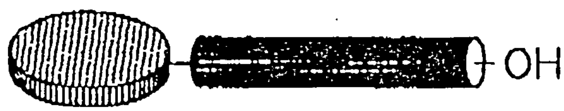
Rewopal MPG 40 is a phenolmonoglycol ether. No components of the mixture above contains antimicrobial agents as mentioned in *Sipos*. To eliminate some effects of the basic composition the MIC (minimal inhibiting concentration) values of the blank value have been set equal to 100%. Every effect of the alcohols tested has been measured against this blank value. The test method for the MIC values is disclosed in the present application.

The MIC values in relation to the blank value are given in brackets in the following text (only a different presentation of the values shown in the table page 17 of the PCT/EP95/05068). Therein for example '(50%)' means that this compound shows in average 50% of the minimal inhibiting concentration than the blank value do and therefore is somewhat active. 25% is more active; 12.5% is the most active in this comparison; bold values are compounds claimed in the present application.

Average means average of the results against s. aureus, c. albicans, p. funi, a. niger (without p. vulgaris), see also table attached.

1. Basic results

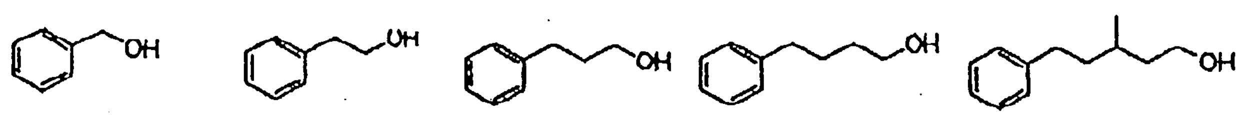
The active structure has the following type:



Lipophilic unit Benzene groups or alicycles	***rigid spacer unit*** $(CH_2)_n$ n=3-5 also C=C	***Hydrophilic alcohol function***

The MIC values correlate with the lipophily of the alcohols tested due to structural reasons. An optimum of activity is found for the structure shown above. Some examples will explain this:

1.1 Substituted n-phenyl alkanols



a	b	1	c	d
(47%)	(50%)	(50%)	(25%)	(13%)

Results: the activity increase with longer chain. 3-methyl-5-phenyl-pentanol (**d**) with n=5 is in this homologue group the most active compound. Tests were not done with n>5.

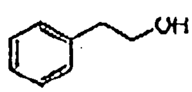
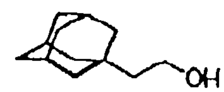
Explanation: the results are in accordance with the above mentioned general activity structure. Important is a significant distance between the lipophilic benzene ring and the hydrophilic OH group.

1.2 Alicycles instead of benzene rings

e f g

(38%) (25%) (4%)

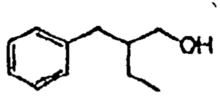
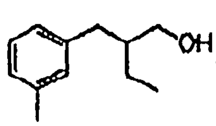
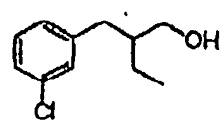
b e h i

(50%) (38%) (28%) (14%)

Results: the activity of the alicyclic homologues increase with chain length (**e**, **f**, **g**). The exchange of the benzene ring by alicyclic structural units as cyclohex(en)yl, norbornyl or adamantyl groups results in increasing activity (**b**, **e**, **h**, **i**)

Explanation: these results confirm the importance of the lipophily of the 'lipophilic unit' of the activity structure mentioned under 1.) instead of the topology. The adamantyl unit is from higher lipophily due to the hydrogen atoms pointing outside the ball sphere. The homology of the cyclohexyl rings is only important to gain a significant distance for the 'spacer unit'.

1.3 Substituents in the benzene ring

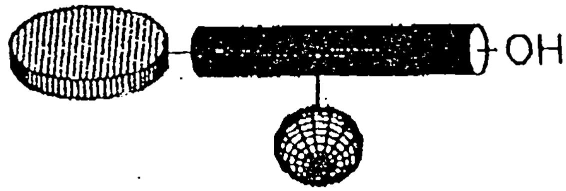
1 4 5 6

(50%) (19%) (17%) (11%)

Results: the activity is increased from 3-phenyl alcohol (**1**) to the 2-methyl substituted alcohol **4** and from **4** to the benzene ring chlorine substituted compound **6**.

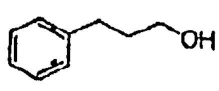
2. Optimized structure and activity has a lipophilic branch within the rigid spacer unit

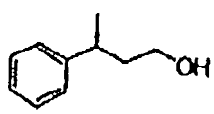
The optimized structure has a branch within the spacer unit

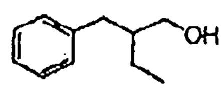


Lipophilic unit Benzene groups or alicycles	*rigid spacer unit* $(CH_2)_n$ n=3-5 also C=C	*Hydrophilic alcohol function*

2.1 Position of alkyl substitution within the spacer unit


1
(50%)

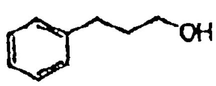
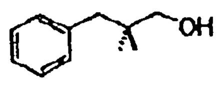
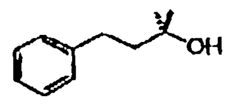

j
(50%)


4
(19%)

Results: the activity is increased from 3-phenyl alcohol (**1**) by alkyl substitution in 2-position as shown for 2-methyl-3-phenylpropanol (**4**). No impact is seen with substitution at the position neighboured to the phenyl group as shown for 3-methyl-3-phenylpropanol (**j**).

Explanation: the (lipophilic alkyl) substituents are more effective if they are in the middle of the spacer unit (CH_2 chain). Best position for activity is the ß-position to the benzene ring as shown for **4**.

2.2 Position of dialkyl substitution within the spacer unit

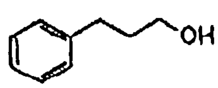
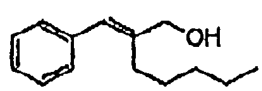
  

1	2	k
(50%)	**(28%)**	(38%)

Results: in the case of dimethyl substitution the 2,2-disubstitution show impact on activity (2,2-dimethyl-3-phenylpropanol **2**) while 1,1-substitution does not as shown for 1,1-dimethyl-3-phenylpropanol (**k**).

Explanation: the dialkyl substitution is more effective if they are in the middle of the spacer unit (CH_2 chain), especially in the ß-position (**2**). Much less activity is found for γ-position (**k**).

2.3 Rigidity of spacer unit and substitution within the spacer unit

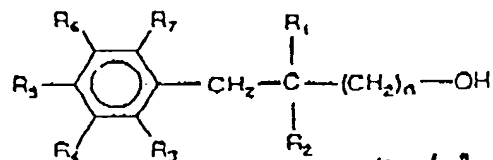
1	7	8
(50%)	**(25%)**	**(22%)**

Results: if the spacer unit is more rigid, together with a substitution in ß-position more activity is found (**7**,**8**).

Explanation: A *rigid* spacer unit as shown in **7** and **8** is effective if there are lipophilic alkyl substituents in the middle of the spacer unit. The α-position to the benzene ring ensures an optimum of rigidity

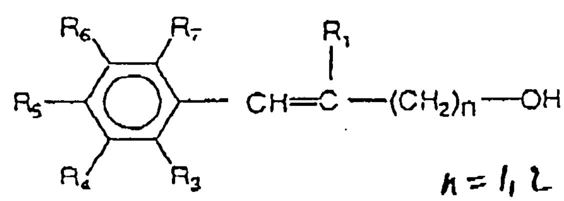
3. Structure of compounds claimed in the present application fit optimized structure.

The optimized structure of the screening tests result to the formula **I** and **II** as claimed in the present application.



$n = 1, 2$

I



$n = 1, 2$

II

Lipophilic unit Benzene groups	***rigid spacer unit*** $(CH_2)_n$ n=3-4 also C=C	***Hydrophilic alcohol function***

The **lipophilic unit** in both formulas is represented by the benzene ring itself or with substituents R_3 to R_7, wherein R_3 to R_7 are selected from lipophilic groups like alkyl, alkenyl, alkinyl, halogen, nitrile or thiocyanate.

The **spacer unit** is represented by a C_3 (n=1) or C_4 (n=2) alkyl chain which fits the minimum distance between the lipophilic unit and the hydrophilic alcohol fuction as demonstrated in 2.1.
The branch is best positioned in the middle of the CH_2 chain as shown in 2.1. The best position also for n=2 is the ß-position of the benzene ring.
The branch substituents are lipophilic alkyl chains either one substituent or in formula I disubstitution by alkyl chains.
In terms of rigity there is a benefit from a C=C double bound as claimed for formula **II**. The α-position to the benzene ring ensures an optimum of rigidity.

The **hydrophilic alcohol function** is essential for activity.

MIC (minimal inhibition concentration) values of biocidal alcohols

Data in µmol/100 ml test solution

Formula no.	S. Aureus	P. Vulgaris	C. Albicans	P. Funi	A. Niger	Average*
Blank value	2.500	1.250	1.250	625	1.250	100%
1	1.250	625	625	313	625	50%
2	313	313	313	313	313	28%
3	2.500	2.500	625	156	156	47%
4	313	2.500	313	156	156	19%
5	156	2.500	313	156	156	17%
6	156	2.500	156	78	156	11%
7	625	2.500	313	156	313	25%
8	39	1.250	313	313	156	22%
a	1.250	2.500	625	313	625	50%
b	1.250	625	625	313	625	50%
c	625	625	313	156	313	25%
d	78	2.500	156	156	156	13%
e	625	1.250	625	313	313	38%
f	313	313	625	156	156	25%
g	78	2.500	39	39	39	4%
h	313	2.500	313	313	313	28%
I	156	2.500	156	156	156	14%
j	1.250	625	625	313	625	50%
k	625	625	625	313	313	38%

* *Calculation:* ($MIC_{S.aureus}$alcohol/$MIC_{S.aureus}$blank value + $MIC_{C.albicans}$alcohol/$MIC_{C.albicans}$blank value + $MIC_{P.funi}$alcohol/$MIC_{P.funi}$blank value + $MIC_{A.niger}$alcohol/$MIC_{A.niger}$blank value)/4 (without P.vulgaris)

Translation

R.Berscheid, M. Nieger, F.Vögtle

Concave Triphenylmethane Dyestuffs

Filed April 13th, 1992

Abstract

Triply- and doubly-brigded as well as dye molecules (**2,18** and **11**) connected by a single chain were prepared including the corresponding phenolphthalein and fluorescein derivatives (**24a-c**). The concave orange-coloured triphenylmethane dye **2** exhibits solvatochromic and halochromic effects different to nonmacrocyclic reference substances. The triphenylmethane dyes are reversibly transferred to the corresponding uncoloured macrocycles by changing the pH. The structures of **9**, **22b** and **22c** were confirmed by X-ray analyses.

Introduction

Shortly we reported the synthesis of the first representatives of macrobicyclic dyestuffs **2** on the basis of triphenylmethane, which are a new type of dyestuffs wherein typical chromophores surrender resp. form a large molecular cavity[1]. This means that the chromophoric group and the molecular cavity are 'joined' together. If a guest molecule can be included into the cavity a change of the color of the dyestuff should be expected corresponding on the shape of the guest molecule similar as it is observed with chromoionophores and small metal ions as their guests[2]. Though the triphenylmethane basic structure and their pH dependant change of color are known since years[3], similar potential 'pH depending switchable' structure units haven't been integrated into host molecules with large cavities[4].

Scheme 1. "Switchable' cavity structure in macrobicyclic triphenylmethane compounds due to the change of pH; top: the example of 1/2; bottom: schematic (ideal model).

1 (colorless) ←→ **2** (orange)
A (colorless) ←→ B (orange)

The threefold bridging of two triphenylmethane units leads to the macrobicyclic host compounds **1** and **2** (scheme 1). Controversy to the trivial dehydroxylation of the nonbridged triphenylmethanols **3** to **4** (scheme 2) the bis-dehydroxylation of **1** to **2** leads to a change of the shape of the molecule's cavity (as shown in scheme 1 model like) and to two different sites of the triphenylmethane chromophores.

While for the outer area of the concave dyestuff molecule **2** similar interactions are expected than for the non-bridged cation **4** the inner side should lead to modified interactions due to the bridging which is not existent in **4**. The cavity compound **2** should be able to include small guest molecules based on our experiences [5] and moreover the changes due to such an interaction should be visible by a shift of the longest waved absorption bandwidth of the host; in the case of the non-bridged compounds **4** such additional shifts should not be existent.

Scheme 2. Trivial halochromy of the non-bridged reference system **3**/**4**[3].

3 (colorless) ←→ **4** (red)

To proof these thoughts we investigated the concave dyestuff **2** on solvatochromic and halochromic effects and on the influence of guest molecules on the longest waved absorption bandwidth, each in comparison to the non-concave reference compound **4**.
To get a clear overall picture we additionally synthesized the onefold-bridged reference compound **6**, the monocyclic dication **11** as well as the triply-bridged monocation **18**. For the syntheses of monocyclic dications **24** which are structural related to **11** a uniform synthesis strategy was developed.

1. Syntheses

(not translated)

2. Solvatochromic and halochromic effects

The rigid three-fold bridging of the triphenylmethane system in the macrobicycles 1 and 2 leads to a build-up of a comparable large and rigid cavity. Due to that fact the two rigidly connected triphenylmethyl cations in **2** are not only interacting by electrostatic effects but also by light absorption effects. So the light absorption of **2** is – compared to the open chain connected system **6** and the macromonocyclus 11 - changed significantly. Table 1 compares the λ_{max} values of the longest waved UV/Vis absorption bandwidth of the dyestuffs mentioned before.

Table 1. Solvatochromic effects of the concave dyestuff 2 compared to the colored reference compounds **4**, **6** and 11 (UV/Vis absorptions in nm)

		λ_{max} values of the longest waved UV/Vis absorption

		bandwidth in solvents				
	No. of bridges between color centers	CH_3CN	CH_2Cl_2	1,2-Dichloroethane	THF	$CHCl_3$
4	0	481	486	487	487	489
6	1	483	487	488	488	490
11	2	484	483	485	488	n. solutable
2	3	481	476	479	487	n. solutable

The comparison shows that compared to the open chained compound **4** only the bicyclic compound **2** has larger hypsochromic shifts (up to 10 nm); additionally an irregularity of the trend of the λ_{max} values is observed with the solvent acetonitrile. Compared to dichloromethane for the open chained **4** and the onefold bridged dication **6** we find hypsochromic shifts if the solvent acetonitrile is used (-5 resp. -6 nm), while compound **11** which has a molecular niche and compound **2** which has an internal molecular cavity show increasing bathochromic shifts of the longest waved UV/Vis absorption bandwidth (+1 resp. +5 nm). This behaviour could be explained by an increasing ability of the (multiple) bridged dyestuffs to include acetonitrile within the molecular cavity inbetween the two triphenylmethane dyestuff centers which cause for the macrobicyclus **2** - due to a molecular model study - a levelling of the propeller like drilled *para*-phenylene rings and therefore leads to a bathochromic shift.
Table 7. Influence of 'guest molecules' on the longest waved UV/Vis absorption [nm] of the colored host compound **2** compared to the non-bridged reference compound **4** measured in dichloromethane. + means a bathochromic shift of the longest waved UV/Vis absorption band of **2** against **4**; - means hypsochromy.

UV/Vis experiments in dichloromethane seems to support this hypthesis. So the incremental addition of acetonitrile to a solution of the concave dyestuff **2** in dichloromethane leads to a bathochromic shift of the λ_{max} values up to 3 nm, whereas the open chained reference compound **4** shows no shift. Also other unsatured flat 'guest compounds' generate bathochromic shifts of a similar size in the case of **2** but not with **4** (Scheme 7).
The observed UV/Vis absorption shifts are small but reproduceable. Also further exeriments (first addition of *p*-xylene: no shift; then addition of *o*-xylene: $\Delta \lambda_{max}$ = +2.5 nm) confirm the results of the bathochromic shift. It should be carefully considered that all experiments have been made in dichloromethane wherein the investigated neutral molecules can be solved very well. Solvophobic

effects for the building of complexes are not merely existant. For symmetric polymethines the compounds 2 and 4 do count there are reported only very small solvatochromic effects[8].
For flat aromatic 'guest molecules' a dependency of the bathochromic shifts due to the shape and the electron density is reported. Within the homologues series of alkylarenes the largest values are observed for multiple substitution like *o*- and *m*-xylene as well as mesitylene. p-xylene which has an unfavourable substitution pattern for the inclusion into the C_3 symmetric molecular cavity shows <u>no</u> shift of the longest waved UV/Vis absorption (see scheme 7).
The electron rich methoxy substituted arenes lead to the largest bathochromic shifts of **2** compared to **4** as expected. The hypsochromic influence of nitrobenzene is contradictionary to the following explanation of the effects but might be caused by an donor acceptor interaction of the nitro group to the alkyne groups of the macrobicycle **2** which is not possible with the reference compound **4**.
To investigate the halochromy different salts were added in a defined amount to the dichloromethane solution of the dyes. After mixing a slow change of color is observed which has reached the intensity maximum after one minute latest.
The observed shifts in color can be caused by flattening of the propeller like drilled benzene rings (bathochromy by enlarging the electronic π system) or by 'bulging' of the triphenylmethane chromophor either as described bottom.

Possible hypsochromic shift ← **2** → possible bathochromic shift

On the other hand it might be true, that the inclusion of a neutral molecule into the cavity of the concave dyestuff **2** – in analogy to the situation in a capacitor – influence the electric dipol moment of the two chromophor units of **2** and therefore cause a shift in light absorption. The guest molecule than behaves like an isolator in a dielectricum.
Further investigation for the interpretation of the effects described above are underwent actually[9].

3. X-ray analyses

(not translated)

Experimental part

(not translated)